GROUNDWATER POLLUTION MICROBIOLOGY

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MICROBIAL POLLUTANTS: THEIR SURVIVAL AND TRANSPORT PATTERN TO GROUNDWATER

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4.1. INTRODUCTION

The persistence and transport of bacteria and viruses in the subsurface is an area of major interest to those concerned with public health. Almost half of all waterborne diseases are caused by contaminated groundwater (1). In order to develop adequate guidelines for the placement of waste disposal sites and drinking-water wells, information is needed on the fate of pathogenic microorganisms in groundwater.

The fate of pathogenic bacteria and viruses in the subsurface is determined by their survival and retention by soil particles. Both survival and retention are largely determined by the three factors shown in Fig. 4.1. Climate controls two

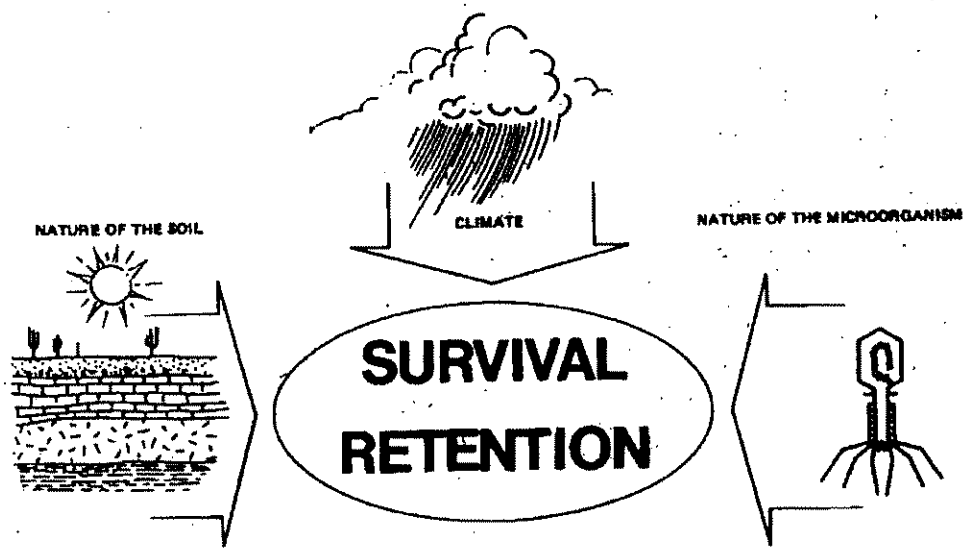

Figure 4.1. Factors affecting microbial fate in the subsurface.

important factors in determining viral and bacterial survival: temperature and rainfall. The survival of microorganisms is greatly prolonged at low temperatures; below 4°C they can survive for months or even years (2). At higher temperatures, inactivation or die-off is fairly rapid. In the case of bacteria (and probably viruses), the die-off rate is approximately doubled with each 10°C rise in temperature between 5°C and 30°C (3). Above 30°C temperature is probably the dominant factor determining virus survival time. Rainfall mobilizes previously retained bacteria and viruses and greatly promotes their transport to groundwater. Several studies have shown that the greatest degree of drinking-water well contamination occurs after periods of heavy rainfall (4–6).

The nature of the soil also plays a major role in determining survival and retention. Soil properties influence moisture-holding capacity, pH, and organic matter—all of which control the survival of bacteria and virus in the soil. Other soil properties such as particle size, cation-exchange capacity, and clay content influence retention. Microbial resistance to environmental factors varies among different species as well as strains. Bacteria are believed to be removed largely by filtration processes while adsorption is the major factor controlling virus retention (2). The following sections are a summary of the factors currently believed to influence microbial persistence and transport in the subsurface.

4.2. BACTERIAL MOVEMENT THROUGH SOILS

4.2.1. Filtration

The straining or filtration of bacteria at the soil surface is a major limitation in their travel through soils. When suspended particles, including bacteria, accumulate on the soil surface, these particles become the filter as water passes through the soil (7). Such a filter is capable of removing even finer particles by bridging or sedimentation before they reach and clog the original soil surface. This phenomenon will, in fact, be dominant if only a portion of the suspended particles are larger than the pore openings. As soon as a few such particles have accumulated, they become the straining surface for finer particles (7).

In studies in which *E. coli* suspended in distilled water were allowed to percolate into sand columns, Krone et al. (7) found that after the first arrival of bacteria, the concentration in column effluents continued to rise until a maximum was reached, after which it fell. This suggests that accumulating bacteria at the soil surface enhances removal by straining. When the capacity for removal by straining has been satisfied along the length of the column, sedimentation alone is operative and the concentration of organisms in the effluent becomes constant.

This same effect is seen during the land application of domestic sewage when repeated cycles of flooding and drying of infiltration basins are used (8). For example, at the Flushing Meadows Project near Phoenix, Arizona, treated

sewage effluent is spread into basins underlaid with loamy sand. The greatest number of fecal coliforms are observed after the start of each new inundation period when nearly infiltrated water arrives at the bottom of sampling wells. Afterwards a general decrease in values occur (see Fig. 4.2). A similar phenomenon occurs when water containing microorganisms is pumped into recharge wells.

Mat formation may be more significant in retaining bacteria in some types of discharges, such as septic tank liquors. The high solids content of these discharges can act to retain significant numbers of bacteria. Mats adjunct to septic tank drain fields have been observed to retain as much as 99.9 percent of the original coliform population over a distance of less than a foot (9).

Studies using sandy soils of various effective porosities indicate removal of bacteria from a liquid percolating through a given depth of soil is inversely proportional to the particle size of the soil. The greatest removal of bacteria occurs on the surface mat (top 2–6 mm) that forms on the soil.

The size and shape of microorganisms also plays a role in their removal by filtration. Bacterial movement through saturated sandy soil columns can be treated in terms of gel permeation chromatography, where the pore volume available for cell movement is considered. If there is no adsorption, larger cells should move faster than smaller cells in a soil column or at a given distance from a subsurface injection point. This is due to the fact that soil capillaries are of various sizes and microorganisms move more freely through the larger pores. Bitton et al. (10) observed this effect, noting that a large encapsulated strain of

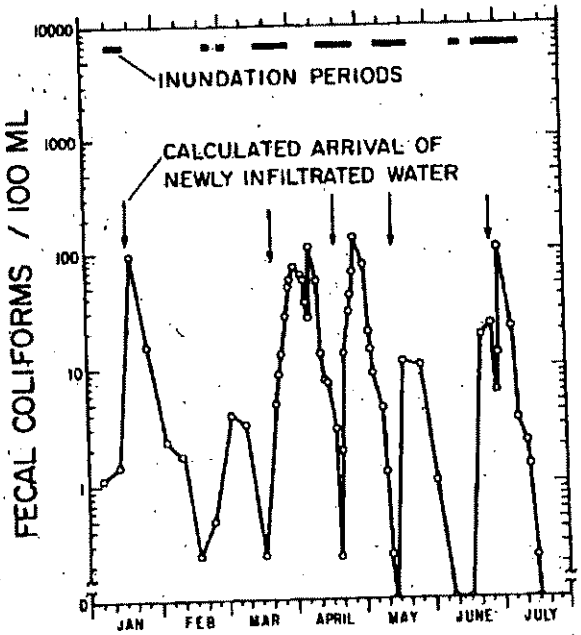

Figure 4.2. Fecal coliforms in well water during multiple flooding and drying cycles of treated wastewater (8). Courtesy of the Water Pollution Control Federation.

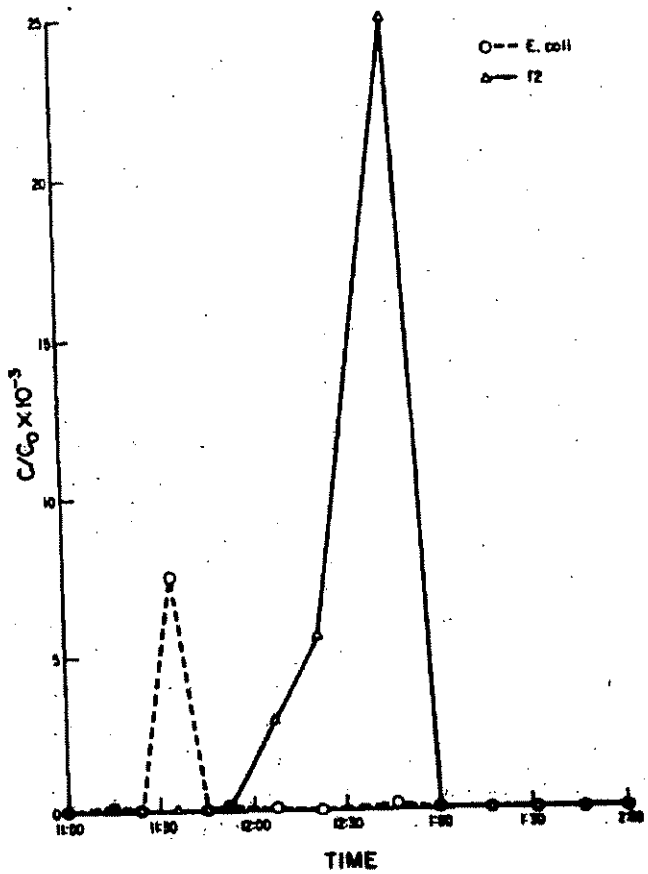

Figure 4.3. Breakthrough curves for *Escherichia coli* and coliphage f2 in a tracer test. Organisms were fed simultaneously into a 300-gpm flow of water that was being injected into a well. The figure shows the concentration of organisms collected from an observation well 500 feet away. The quantity C_0 is the concentration of the tracer in the injected water and C is the concentration measured in the observation well.

Klebsiella aerogenes moves faster than a smaller noncapsulated strain. The effect can also be seen in Fig. 4.3 which illustrates the arrival of the bacterium *E. coli* and coliphage f2 in an observation well 17 m from the point of injection. The larger *E. coli* arrived at the well first, followed by the much smaller coliphage f2.

4.2.2. Adsorption

Adsorption is also a factor in the removal of bacteria by soil. Factors that reduce the repulsive forces between two surfaces, such as the presence of cations, would be expected to allow closer interaction between them and to allow adsorption to proceed. A more detailed explanation of mechanisms involved in adsorption is found in the section on viruses. The very small size of clays, their generally platy

shapes, and the occurrence of large surface area per given volume make them ideal adsorption sites for bacteria and viruses in soils. Thus, adsorption phenomena play a more important role in the removal of microorganisms in soils that contain clays (11).

Goldshmid et al. (12) found that filtration efficiency of coliforms through sand is higher when the medium is tap water rather than distilled water. No bacterial removal occurred when triple-distilled water was used. Increases in the cation concentration and valence resulted in increases in the retention efficiency. The retention efficiencies were similar to those predicted from the Shulze–Hardy laws, which describe the effect of the addition of electrolytes on the stability of lyophobic colloids. In the presence of oxidation pond effluents, more cations were found to be necessary to achieve the same retention efficiency as with tap water. In addition, a decrease of pH from 9.3 to 3.9 increased the retention efficiency, again in agreement with adsorption phenomena. The reversibility of this phenomenon was shown when the ionic concentration of column influent was reduced and a corresponding increase in the number of coliforms in the column effluent occurred.

Other studies have demonstrated that common cations in solution will affect bacteria adsorption to soils (13). Certain metallic cations (Fe^{3+}, Cu^{2+}, Zn^{2+}) at concentrations not uncommon to soils have been shown to enhance the removal of bacteria. The common fertilizer ion NH_4^+ also enhances the removal of bacteria. The anions Cl^-, SO_4^{2-}, and NO_3^- have little effect on adsorption (13). Low soil pH also enhances bacterial retention (10). Soluble organics may also compete with bacteria for adsorption sites onto the soil, thereby reducing bacterial adsorption (14).

Rainfall will effect bacterial retention by lowering ionic concentration and increasing infiltration rates. Several surveys have indicated that rainfall and well depth are related to microbial groundwater quality. Studies in Washington indicate that shallow drinking-water wells average median coliform values of 8 MPN/100 ml with an average depth of 9.4 m (31 ft) while deep wells with an average depth of 153.3 m (503 ft) average 4 MPN/100 ml (14). They also observed that virtually all bacterial contamination coincided with the periods of heaviest rainfall. Brooks and Cech (15) observed in rural eastern Texas that practically all dug wells with depths of 15 m (50 ft) or less were positive for either fecal coliforms or fecal streptococci. While the presence of fecal bacteria was much less common in deeper wells, wells as deep as 80 m (250 ft) were positive. Increased levels of bacterial contamination of drinking-well water after periods of rain have been noted in several studies (4,5,6,16). It was also noted that while an increase in coliform bacteria appears almost immediately after periods of heavy rainfall in shallow wells, the increase did not occur until two weeks later in deeper wells (17). Thus, any satisfactory study of well water quality should include sampling during periods of highest rainfall. Sandhu et al. (18) found that basic well design and construction had little effect on the extent of microbial pollution in their study area.

4.3. SURVIVAL OF ENTERIC BACTERIA IN SOIL

At the turn of the century, it was found that eating raw vegetables grown on soil fertilized with raw sewage resulted in outbreaks of typhoid fever. As a result, the survival of enteric bacteria in soil systems has been extensively studied. There are several major reviews on the survival of enteric bacteria in soil (17–21), but we only consider those factors that affect the length of survival of these bacteria. Most enteric bacterial pathogens die-off very rapidly outside of the human gut, whereas indicator bacteria such as *Escherichia coli* persist for longer periods of time. Survival times among different types of bacteria vary greatly and are difficult to assess without studying each type individually. In most cases, it appears that 2–3 months are sufficient for the reduction of pathogens to negligible numbers once they have been applied to the soil. However, survival times of as long as five years have been reported (19). Factors known to influence bacterial survival in the soil are listed in Table 4.1.

4.3.1. Moisture

A major factor in determining the survival of bacteria in the soil is moisture. Young and Greenfield (22) showed that moisture was a factor in the viability of *Escherichia coli* in soils. Beard (23) stated that moisture was the most important determining factor in the survival of *Salmonella typhosa*. Bacterial survival was determined in various types of soil exposed outdoors in clay flowerpots. Survival in all types of soil tested was found to be greatest during the rainy season. In sand, where drying was rapid due to its low moisture-retaining power, survival time was short—between 4 days and 7 days during dry weather. In soils

Table 4.1. Factors Affecting Survival of Enteric Bacteria in Soil[a]

Factor	Comments
Moisture content	Greater survival time in moist soils and during times of high rainfall
Moisture holding capacity	Survival time is less in sandy soils with lower water-holding capacity
Temperature	Longer survival at low temperatures; longer survival in winter than in summer
pH	Shorter survival time in acid soils (pH 3–5) than in alkaline soils
Sunlight	Shorter survival time at soil surface
Organic matter	Increased survival and possible regrowth when sufficient amounts of organic matter are present
Antagonism from soil microflora	Increased survival time in sterile soil

[a]Modified from Ref. 2.

that retain a high amount of moisture, such as loam and adobe peat, the organisms persisted longer than 42 days.

Bouma et al. (24) have suggested that survival data for fecal organisms could be compared with soil-moisture characteristic curves, and hence, the distance of soil filtration necessary for removal be defined as a function of moisture content. In studies on the survival of enteric organisms in septic tank discharges, Parker and Mee (25) concluded that under field conditions survival is not likely to be limited by moisture in coarse sands. They felt that enough moisture was available in the drainfields to prevent moisture from playing a significant role and thus could not be used to predict survival under normal field conditions.

4.3.2. pH

Beard (23) also found that the death of *S. typhosa* was very rapid in peat soil with a pH between 3 and 4. Kligler (26) found that moist, slightly alkaline soils were most favorable for the survival of *S. typhosa.* Cuthbert et al. (27) inoculated various peat (pH 2.9–4.5) and limestone (pH 5.8–7.8) soils held in the laboratory with *E. coli* and *Strep. faecalis.* They found that both organisms could persist for several weeks in the limestone soils, but would die out in a few days in acid peat soils. They felt that the low pH could not only adversely affect the viability of the organism but the availability of nutrients and the action of inhibiting agents.

4.3.3. Sunlight

Beard (23) found that sunlight exerted a definite lethal action on typhoid organisms at the soil surface. Van Donsel et al. (28) reported a greater die-away of both *E. coli* and *Strep. faecalis* when added to soil plots exposed to direct sunlight rather than a shaded area. These results are not surprising, because the ultraviolet light present in sunlight is known to be bactericidal.

4.3.4. Temperature

Cold temperatures favor the survival of most microorganisms and enteric bacteria are no exception (29). *S. typhosa* may survive as long as 24 months at freezing temperatures (23). Mirzoev (30) points out that in areas with prolonged winters, for example, the Russian Arctic, the processes of soil self-disinfection are slowed down or suspended. He showed that low temperatures (down to −45° C) were very favorable for the survival of dysentery bacilli, which he was able to detect 135 days after it had been added to the soil. Van Donsel et al. (28) found that a 90 percent reduction in the number of fecal coliforms took 3.3 days in the summer and 13.4 days in the winter in exposed soil plots.

In contrast to these observations, Kibbey et al. (31) found that any length of freezing conditions was highly lethal to *Streptococcus faecalis*, and that numerous freeze–thaw intervals were more destructive than one extended freeze. Organisms surviving freezing declined more rapidly because of greater cellular damage caused by the freeze–thaw cycles. Soil moisture, the nature of the organism, and other factors greatly influence the effects of freezing on bacteria in soils.

4.3.5. Organic Matter

The frequent addition of broth culture fluid to soil has been found to increase the survival of *S. typhosa* (19). Under field conditions, it has been found that some aftergrowth of *E. coli* and *Strep. faecalis* can occur, particularly after wet weather (28). The survival of fecal coliforms is greatly extended in organic soils over that observed in mineral soils (32) and regrowth of *Salmonella typhimurium* and *E. coli* has been observed in buried feces (33). The extended survival and growth in organic soils may be due not only to the presence of organics but to the high moisture-holding capacity of these soils (32).

4.3.6. Other Microorganisms

Soil moisture, temperature, pH, and the availability of organic matter can also indirectly influence the survival of enteric bacteria by regulating the growth of antagonistic organisms (28). Bryanskaya (34) showed that actinomycetes in soil were capable of suppressing the growth of salmonella and dysentery bacilli. In addition, the longer survival time of enteric organisms after inoculation into sterilized soil as compared to unsterilized soil found by a number of workers (19) indicates that antagonism is an important factor. Tate (32) observed that the protozoan population of a muck soil increased dramatically after the addition of *E. coli* and suggested that soil protozoa could play a significant role in the decline of these organisms in these soils. Since it is evident that enteric bacteria are capable of utilizing nutrients found in nature, it could be argued that competition by the natural soil microflora is in large part responsible for their eventual disappearance from the soil.

4.4. VIRUS MOVEMENT THROUGH SOILS

Concern over public health risks associated with land disposal of domestic wastes that contain human and animal pathogens has been raised. Among these risks is the potential for viruses to be transported through soils and thus to possibly contaminate groundwater supplies. On many occasions, epidemics of

infectious diseases were connected to the consumption of contaminated groundwater (this topic is thoroughly reviewed in Chapter 7). This may be due to the fact that viruses may travel through fissures and fractures within the substratum. On many other occasions, viruses were not detected in groundwater beneath sites used for land application of wastewater effluents and residuals (see Chapter 9 for more details). Thus, the public health risks associated with land disposal of human and animal wastes can be evaluated only through a critical review of the various factors controlling the transport pattern of viruses through soils (2, 35–39). Knowledge concerning virus movement through soils has been gained through monitoring land application sites and through batch and column experiments under laboratory conditions.

Batch and column experiments have been widely used to understand the transport pattern of viruses through soils. These studies have been criticized because of the lack of standardization in experimental conditions and because column experiments do not always simulate the natural field conditions (37). However, valuable information has been gained through these studies since many of the factors controlling virus transport through soils have been identified. These factors include soil type, pH, organic matter, cations, flow rate, degree of pore saturation, and virus type.

4.4.1. Soil Type

Viruses are retained by soils mainly through adsorptive phenomena. The adsorption of viruses to soils generally conforms to Langmuir and Freundlich-type isotherms (40–44). Most often, the slope of the Freundlich isotherm is close to unity, which means that the percent virus removal by soils is theoretically independent of virus concentration. Soils offer a large number of sites for viruses to be adsorbed. Moore et al. (43), using Ottawa sand, showed that 2.2×10^6 viruses adsorbed/g sand. They also reported that only 1 percent of the sand surface was covered with viruses. This indicates that soils are potentially efficient in virus binding. Soils are complex environments, and qualitative changes in their textural components (sand, silt, clay, organic matter, iron oxides) undoubtedly influence their sorptive capacity toward viruses. Moore et al. (43) have examined 34 soils and minerals for their ability to retain poliovirus. Virus adsorption varied from 16–79 percent for a muck soil to 99.99 percent for a magnetic sand. The poor adsorption to the muck soil confirms earlier studies (45, 46).

It is generally agreed that fine-textured soils retain viruses more effectively than sandy soils, since the soil clay mineral fraction displays a high sorptive capacity toward viruses as a result of its high surface area and ion-exchange capacity. Following the examination of nine soils from Arkansas and California, it was shown that virus adsorption increased with the clay content and the specific surface area of the soil (41). Koya and Chaudhuri (47) reported that, among all the soils studied, a lateritic soil (32.5 percent clay) was the most

efficient in retaining viruses. Iron oxides, particularly magnetite, also display a high affinity for viruses (48, 49). A magnetic sand and hematite proved to be the most effective virus adsorbents (43). It thus appears that soil iron oxides can increase the ability of soils to retain viruses. Finally, a recent investigation has shown a highly negative correlation between virus adsorption and the capacity of soils to bind a cationic polymer, PDADM (Polydiallyldimethyl ammonium chloride). It was suggested that the ability to bind the polymer could serve as an indicator of the extent of viral adsorption (43).

4.4.2. pH

Most viruses behave as proteins, the net charge of which is dependent on the pH of the suspending medium. As the pH increases, there is an increase in the ionization of carboxyl groups and a decrease in the ionization of amino groups. Consequently, the net charge of the virus particle is negative at pHs above neutrality. Major components of soils (sand, clay minerals, organic matter) are also negatively charged at pHs above 7. Electrophoretic mobility studies have shown that a common soil clay mineral, montmorillomite, is negatively charged at pH between 4.5 and 10.5. Muck soils also have a high negative charge (50). It is tempting to conclude that at alkaline pHs virus adsorption will be at a minimum whereas at acid pHs virus adsorption to soils will reach its maximum. The relationship between virus adsorption and pH is not clear-cut, however, because of many complicating factors. The pH of the soil, as conventionally measured, does not reflect necessarily the pH at the surface of soil colloidal particles such as clays. Various soil components (clay, sand, oxides of Al and Fe) display different isoelectric points. There is also a lack of information on the isoelectric points of more than 100 viruses that occur in wastewater or groundwater. So far, we know that the isoelectric point varies with the virus type and strain (50–53). Even more complicating is the fact that a given virus may display two isoelectric points. For example, poliovirus 1 has two isoelectric points at pH 4.5 and 7.0 (52) and poliovirus 2 displays two isoelectric points at pH 4.5 and 7.5 (50). The factors responsible for the passage from one form to another are not known. It is possible that some other soil factors (e.g., cations or humic acids) may also influence the net electric charge of viruses. The previous discussion can help us understand the confusing trends reported in the literature. Some researchers have reported an increase in adsorption as the pH decreases (40, 41, 51) while others found no correlation between pH level and adsorption (43).

4.4.3. Conductivity of the Percolating Water

Conductivity is a measure of the ionic strength of a solution and is expressed in mmhos/cm or μmhos/cm at 25° C. It is generally agreed that the concentration

and species of cations in the percolating water affects the extent of virus adsorption to soils. Early experiments with bacterial phages showed that adsorption to soil increased with the cation concentration of the percolating solution (41). Divalent cations (e.g., Ca^{2+}, Mg^{2+}) were very efficient in virus adsorption to a sandy soil and most of the virions were detected in the top layer of the soil (54). Thus, cations are necessary for reducing the repulsive forces on both the virus and soil particles and allow adsorption to take place. Virus retention by soils is generally greater in the presence of sewage effluents than in distilled water (55). Wastewater effluents have higher conductivity (500–600 μmhos/cm) than distilled water (2–10 μmhos/cm) or rainwater (20–40 μmhos/cm). Rainwater, being of lower conductivity than sewage effluents, may lead to reduced virus adsorption or to virus desorption with the subsequent redistribution of viruses within the soil profile. This phenomenon was well demonstrated via soil core studies under controlled laboratory conditions (46, 55, 56). Landry et al. (57) showed that virus penetration was more extensive in rainwater-rinsed cores than in wastewater-rinsed cores. Moreover, the desorbed viruses may readsorb at greater depths. Heavy rainfall might then remobilize soil-bound viruses with the potential contamination of groundwater supplies (58). However, it now appears that the ability of rainwater to release viruses depends on the soil type, the release being more pronounced in sandy then in clay soils (46, 59). The elution pattern also depends on the virus type and strain. For example, poliovirus 3 and echovirus 6 were mobilized by artificial rainwater whereas echovirus 1 was not affected. The elution pattern of the reference strain of poliovirus 1 differed from that of field and mutant strains (60). The application of these concepts to virus management during land application of wastewater effluents will be discussed by Lance (Chapter 9)

4.4.4. Soluble Organic Materials

Soluble organic materials are known to compete with viruses and bacteria for adsorption sites. It may then be possible that organics present in sewage may interfere with virus sorption to soils. However, several studies have shown that viruses are well adsorbed to various types of soils in the presence of secondary and even primary wastewater effluents (42, 55, 61). As discussed above, wastewater effluents contain enough salts to overcome any interference by soluble organic matter.

Humic and fulvic acids constitute a category of soluble organic matter that may interfere with virus adsorption to soils. They may increase virus transport through organic soils and, in particular, situations where sewage effluents are discharged into wetlands (45, 62). This phenomenon was confirmed by Bixby and O'Brien (63) who reported that fulvic acids complex MS2 phage and prevent its adsorption to soil. More recently, muck soils were found to display a lower adsorption capacity than mineral soils (43, 46).

4.4.5. Virus Type

During the last two decades great attention has been focused upon the quest for a virus indicator that could adequately simulate the adsorption–elution pattern of all the other enteric viruses. First, researchers focused on bacterial phages since their assay is rapid and inexpensive. This approach was criticized and numerous studies were then undertaken on the adsorption–elution pattern of poliovirus type 1. It is now agreed that poliovirus type 1 adsorbs well to soils. More recently, Goyal and Gerba (64) studied the adsorptive behavior of 27 enteroviruses, a simian rotavirus (SA-11), and 5 bacterial phages. It was found that most of the viruses adsorbed well to soils except for echo 1, echo 12, echo 29, SA-11, and phage f2. Echo 1 and RNA phage f2 were the least adsorbed among all the viruses tested. Others have confirmed the low ability of echovirus 1 to adsorb to soils, organic flocs, and sludges (65, 66). However, others showed that echovirus 1 displayed a high affinity for soils (60). These apparently conflicting findings show that virus adsorption–elution patterns in soils vary not only with virus type but with isolates within the same type. Thus, differences in sorptive ability were noted among natural isolates of echovirus 1 and coxsachivirus B4 (64). On the other side of the scale, poliovirus 1 and T-even phages displayed the highest adsorption to soils (64). This confirms the data of Bitton et al. (62) who showed that poliovirus 1 and T2 phage were equally well adsorbed to soils. It was recently concluded that viruses may be grouped into three categories according to their adsorptive behavior (51). Category 1 contains the poorly adsorbed viruses (echovirus 1, echovirus 11, coxsackievirus B4, ϕX174, MS2). Category 2 includes the highly adsorbed viruses (poliovirus 1, echovirus 7, coxsackievirus B3, T2, and T4). Phage f2 was placed in a third category.

This demonstrates that poliovirus 1 cannot serve as an indicator to predict the adsorption–elution behavior of enteric viruses in soils. Virus movement through soils to the groundwater appears to be controlled by the interplay between virus type and strain, soil type, and other known and yet unknown factors. The quest for a suitable virus indicator still continues.

4.4.6. Saturated versus Unsaturated Flow of Water

The degree of saturation of pores within the soil matrix is an important parameter to consider in studies of virus retention by soils. Under saturated-flow conditions, water fills all the pores whereas under unsaturated conditions it flows only through the small pores or is retained as a film around soil particles (see Chapter 2). Thus, unsaturated-flow conditions allow viruses to get closer to particle surfaces. This increases the opportunity for virus sorption to soil. Virus movement through soils was examined mainly under saturated-flow conditions using soil columns. These studies probably underestimated the degree of virus retention by soils because they do not simulate the unsaturated-flow conditions

often prevailing in the field. Therefore, soil columns should be long enough to allow the establishment of an unsaturated zone (see Chapter 2). Soil drying between sewage applications is a useful management practice that can prevent the desorption of virus following rainfall (56).

4.4.7. Downward Flow Rate

The lower the infiltration rate of sewage into soil, the longer the retention of viruses within the vadose zone. This results in more efficient virus removal. Green (67) showed that viruses suspended in septic tanks effluents were retained more efficiently by sand mounds as the flow rate of sewage decreased. More recent studies have shown that virus movement through soils was promoted by increasing the flow rate of the percolating sewage (121–123). Management practices for the control of sewage effluents may be important to the treatment process with respect to viruses and other microbial pathogens (see Chapter 9). The factors that may influence virus transport to groundwater are summarized in Table 4.2.

Table 4.2. Factors that may Influence Virus Movement to Groundwater

Factor	Comments
Soil type	Fine-textured soils retain viruses more effectively than light-textured soils. Iron oxides increase the adsorptive capacity of soils. Muck soils are generally poor adsorbents.
pH	Generally, adsorption increases when pH decreases. However, the reported trends are not clear-cut due to complicating factors.
Cations	Adsorption increases in the presence of cations (cations help reduce repulsive forces on both virus and soil particles). Rainwater may desorb viruses from soil due to its low conductivity.
Soluble organics	Generally compete with viruses for adsorption sites. No significant competition at concentrations found in wastewater effluents. Humic and fulvic acid reduce virus adsorption to soils.
Virus type	Adsorption to soils varies with virus type and strain. Viruses may have different isoelectric points.
Flow rate	The higher the flow rate, the lower virus adsorption to soils.
Saturated versus unsaturated flow	Virus movement is less under unsaturated flow conditions.

4.5. VIRUS SURVIVAL IN SOILS

We have reviewed the various factors controlling the transport of viruses through soils. It is now realized that some of the soil-associated viruses may be desorbed and thus redistributed within the soil matrix following changes in certain characteristics of the soil solution (e.g., ionic properties). This may result in groundwater contamination. Another important aspect of groundwater pollution is the persistence of virus particles in the soil environment. This subject has been extensively reviewed by various investigators (2, 35, 68, 69). We shall limit ourselves to the analysis of the most pertinent factors controlling virus survival in soils. It is worth mentioning at this point that significant contributions were made by phytopathologists as well as insect virologists. Phytopathogenic viruses (e.g., wheat yellow mosaic virus, barley yellow mosaic virus) are known to survive for extended periods in soils (70–72). Clay minerals appear to play an important role in the extended survival of plant viruses (71, 72). Similarly, insect viruses (e.g., nuclear polyhydrosis virus, granulosis virus) persist well in soils (73–75). The above findings have inspired investigators interested in the fate of human and animal viruses in soils. It was, however, essential to learn more about virus detection in soils. Soil-associated viruses can be recovered using a variety of eluents ranging from tryptose phosphate broth (55), minimal essential medium and antibiotics (76), glycine–EDTA (77), beef extract (78, 79), isoelectric casein to nonfat dry milk (78). Quantitative techniques have been developed for virus recovery from soils. These methods include an elution step followed by a concentration step (77, 79).

Soil is a complex heterogenous environment the properties of which may be altered in response to climatic changes and agricultural practices. This means that any alien microorganism (e.g., viruses) introduced into soils may be subjected to a variety of environmental factors or to a combination of factors that are dictated by the interplay between climate, vegetation, soil, and microorganisms. It is also expected that sewage and sludge application to land may alter some of the soil's physical and chemical properties with regard to virus survival (69). However, from an experimental viewpoint it is rather difficult to study the effect of combinations of factors on virus persistence. One can then limit our discussion to an overview of the various factors and speculate when possible on the impact of their interrelationship with respect to virus survival.

4.5.1. Temperature

Temperature is probably the most detrimental factor affecting virus survival in soils as well as in other habitats (68). Temperature obviously affects chemical and biological processes in soils and this may indirectly affect virus decline. Owing to the heterogeneity of viral populations, it is likely that some thermoresistant isolates may survive extreme temperatures in soils (80, 81).

Bagdasar'yan (82) observed that viruses could survive up to 170 days in soil at 3°–10°C and that survival was higher at 3°–10°C than at 18°–23°C. Similar observations were made by Lefler and Kott (54) with regard to poliovirus type 1 and bacteriophage f2 survival in a sandy soil in Israel. The heavy dependence of viral persistence on soil temperature was subsequently confirmed by other investigators (55, 67, 83–87).

Land application of municipal sludge offers many advantages but one should be concerned with the survival of viruses in the sludge–soil mixtures and with the possible contamination of groundwater. It appears that virus survival in sludge-amended soils is controlled primarily by soil temperature and moisture (83, 84, 88, 89). Larkin et al. (76) also observed that poliovirus survival was greater in winter than in the summer in Cincinnati, Ohio. In Denmark, at temperatures ranging from 0°C–10°C, coxsackievirus B3 was shown to survive 161 days in a sludge-amended soil (90). One would expect viruses to survive longer in sludge injected 10–15 cm below the soil surface (89) than in surface-spread sludge (84).

4.5.2. Soil Moisture

Soil desiccation is directly related to temperature and both factors synergetically influence virus survival in soils. Bagdasar'yan (82) was one of the first to report the marked effect of soil moisture on enteroviruses (P1, CB3, E7, E9). Viruses survived no more than 15–25 days in air-dried soil compared to 60–90 days in samples with 10 percent moisture. Similarly, DuBoise et al. (55) reported an increased reduction of poliovirus 1 in a dry sandy soil. Hurst et al. (91) observed that the drying period is important with regard to virus survival in rapid-infiltration basins and that the rate of inactivation depends on the rate of soil moisture loss. Poliovirus 1 could not be recovered from a brown-red sandy soil after 16 days when the moisture dropped from 15 percent to 3 percent (79). Use of labeled viruses has shown that they are truly inactivated rather than irreversibly bound to soil particles (87). Evaporation of soil water may be primarily responsible for virus loss during soil drying (87). However, in controlled laboratory experiments, a survey of the soil characteristics and environmental factors affecting virus survival did not reveal any clear trend regarding the effect of soil moisture (85).

This contradictory finding may be explained by the heterogenous nature of soils. Small variations in texture may lead to drastic changes in the water-holding capacity of soil, thus affecting virus survival. Soil desiccation was one of the most detrimental factors controlling virus persistence in sludge–soil mixtures (84, 88).

4.5.3. Sunlight

It is known that viruses may be inactivated by sunlight in the aquatic environment (92, 93). Sunlight at the soil surface is detrimental and its role is minor in comparison to other environmental factors.

4.5.4. Soil Characteristics

Soils vary in their chemical and physical properties and this, in turn, probably influences the fate of viruses. Early studies (82) did not reveal any clear trend as to the effect of soil type on virus survival. Later, Sadovsky et al. (79) compared poliovirus survival in two soils, a desert alluvial soil and a coastal brown-red sandy soil, but it was difficult to distinguish between environmental factors and soil characteristics. A more expanded study that included nine different soils was undertaken by Hurst et al. (85). It showed that virus survival correlated with the extent of virus adsorption to soil, soil saturation, pH, and exchangeable Al. Viruses are known to survive better in the sorbed state than in suspension (46, 67). The significant correlation observed between virus survival and exchangeable Al may be due to increased viral adsorption in the presence of Al (85). DuBoise and his co-workers (69) speculated that cations and pH affect adsorption, which in turn influences viral stabilization in soils. It has been established that certain cations (Ca, Mg) and pH may increase the thermal stabilization of enteroviruses (94–96). This phenomenon may be of some significance with regard to virus survival in soils.

The two most active components of soil are clay minerals and humic materials. Clay minerals influence the ecology of soil microbial populations as well as microbial pollutants (97, 98). One may speculate that clays increase viral adsorption to soil and thus indirectly increase their stabilization in soils. Viral genome can persist in soils and be protected from nucleases by clay minerals (99–101). In aquatic environments, clays protect viruses from light (92), heat (Schiffenbauer and Stotzky, unpublished), and biological degradation (102, 103). In soils, water may be tightly bound to clays, even though the soil is considered dry and this may influence the survival of microorganisms subjected to desiccation (104). Humic materials probably have an indirect influence on virus survival in soils since they affect the cation-exchange capacity, pH, and moisture retention of soils. No experimental evidence is available, however, to support or refute these speculations.

4.5.5. Biological Factors

As in the aquatic environment (68), it is likely that biological factors may play a role in virus inactivation in soils. There is no clear trend, however, regarding the contribution of soil microflora to virus decline. Some investigators did not observe any effect (76, 82) while others reported a greater virus decline in nonsterile than in sterile soils (46, 85). This reflects the complex and heterogenous nature of soil environment.

4.5.6. Virus Survival Under Field Conditions

Viruses may survive long enough to be detected in groundwater. Enteroviruses have been detected at the surface of soils irrigated with raw sewage in the

U.S.S.R. (105). A field study revealed virus survival for at least 28 days in soil following application of a package treatment plant effluent in a cypress dome in Gainesville, Florida (58). Other field studies confirm the important role played by temperature and soil moisture in virus persistence in soils (79, 91). Similarly, it appears that virus survival in sludge-amended soils is controlled primarily by desiccation and soil temperature (84, 88, 106). During surface application of digested sludge on soils in Pensacola, Florida, it was shown that indigenous enteroviruses were able to survive only nine days after sludge application (107).

4.5.7. Mechanism of Viral Inactivation in Soil

We have discussed the major factors that influence viral persistence in soil but we have yet to learn more about the mechanism(s) of inactivation. It is a fact that desiccation and temperature are the leading factors controlling virus survival. This has led to a study on the structural changes associated with poliovirus decline in soil (108). It was shown that damage to the enterovirus consisted of dissociation of viral components followed by degradation of viral genome. The above investigators also reported that viral RNA was degraded more rapidly in moist than in dry soils. The viral genome may persist in soil and could be protected from nuclease action by soil colloids (69). The factors that may influence virus persistence in soil are summarized in Table 4.3.

4.6. BACTERIAL AND VIRAL SURVIVAL IN GROUNDWATER

We have reviewed the various parameters that control the transport and survival of pathogenic microorganisms in soils. Comparatively little is known about the

Table 4.3. Factors that may Influence Virus Survival in Soils

Factor	Comments
Temperature	One of the most detrimental factors.
Desiccation	One of the most detrimental factors. Increased virus reduction in drying soils.
Sunlight	May be detrimental at the soil surface.
Soil pH	May indirectly affect virus survival by controlling their adsorption to soils.
Cations	Certain cations have a thermal stabilizing effect on viruses. May also indirectly influence virus survival by increasing their adsorption to soil (viruses appear to survive better in the sorbed state).
Soil texture	Clay minerals and humic substances increase water retention by soil and thus have an impact on viruses subjected to desiccation.
Biological factors	No clear trend with regard to the effect of soil microflora on viruses.

Table 4.4. Die-off Rate Constants (day^{-1}) for Viruses and Bacteria in Groundwater

Microorganism	Die-off[a] rate (day^{-1})	Reference
Poliovirus 1	0.046	110
	0.21	112
	0.77	114
Coxsackievirus	0.19	112
Rotavirus SA-11	0.36	112
Coliphage T7	0.15	115
Coliphage f2	1.42	110
	0.39	112
Escherichia coli	0.32	112
	0.36	111
	0.16	110
Fecal Streptococci	0.23	112
	0.24	111
	0.03	110
Salmonella typhimurium	0.13	112
	0.22	111

[a]As $\log_{10} N_T/N_0$, where N_T equals concentration of organisms after 24 hrs and N_0 equals the initial concentration of organisms.

survival of these pathogens in groundwater. Microbial persistence has been studied using flasks incubated under laboratory conditions (109, 110), McFeter-type chambers immersed in flowing groundwater (111, 112), or dialysis tubing suspended directly into the wells (113). Table 4.4 gives the decay rate constants for some viruses and bacteria in groundwater. Data available indicate that bacteria and viruses survive longer in groundwater than in surface waters. For example, the decay rate of poliovirus type 1 in groundwater is 0.0019 hr^{-1} (110) compared to 0.031 hr^{-1} in river water (114) or 0.020 hr^{-1} in seawater (116). Microcosm studies also show that viruses survive longer than indicator bacteria in groundwater (110, 112, 114, 117). There is the need to find suitable indicators of viral contamination since no correlation was found between indicator bacteria and viruses in groundwater (118). Indicator bacteria may grow in groundwater if sufficient nutrients are present. *E. coli* growth was observed during groundwater recharge operations in Israel (119). It was also found that coliform growth occurred during the period between recharge and pumping (120).

It appears, from the above investigation, that more research needs to be done on the persistence of microbial pathogens in groundwaters from various geological formations and depths. A suitable indicator of viral contamination in groundwater should also be found.

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